Examination of Tissue Metal Burdens and Metal-Binding Proteins in the Golden King Crab (Lithodes Aequispina Benedict) from Alice Arm and Hastings Arm, British Columbia

J.A.J. Thompson, M.D. Nassichuk, D.W. Paton,
B.J. Reid, M.A. Farrell

Field Services Branch
Department of Fisheries and Oceans
1090 West Pender Street
Vancouver, British Columbia V6E 2P1

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EXAMINATION OF TISSUE METAL BURDENS AND METAL-BINDING PROTEINS IN THE GOLDEN KING CRAB (LITHODES AEQUISPINA BENEDICT) FROM ALICE ARM AND HASTINGS ARM, BRITISH COLUMBIA

by

J.A.J. Thompson,*[1] M.D. Nassichuk, D.W. Paton[1], B.J. Reid, M.A. Farrell

Water Quality Unit
Habitat Management Division
Field Services Branch
Department of Fisheries and Oceans
1090 West Pender Street
Vancouver, B.C. V6E 2P1

[1] Ocean Chemistry Division
Institute of Ocean Sciences
P.O. Box 6000
Sidney, B.C., V8L 4B2

* Author to whom correspondence should be addressed

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ABSTRACT

Thompson, J.A.J., M.D. Nassichuk, D.W. Paton, B.J. Reid, and M.A. Farrell. 1986. Examination of tissue metal burdens and metal-binding proteins in the Golden King Crab (Lithodes aequispina) from Alice Arm and Hastings Arm, British Columbia. Can. Tech. Fish. Rep. Aquat. Sci. No. 1440.

Gill, muscle and hepatopancreas tissues from the Golden King Crab (Lithodes aequispina Benedict) collected from two sites in Alice Arm and from four sites in Hastings Arm, British Columbia were analysed for burdens of 32 elements by inductively coupled plasma spectrometry. Data for six elements: arsenic, cadmium, copper, lead, molybdenum and zinc were chosen for detailed examination owing to their importance in relation to mine tailings discharge from the AMAX molybdenum facility in Alice Arm. Hepatopancreas tissue from each specimen was also analysed for metal-binding protein content using differential pulse polarography. Molecular weight distribution of the cytosolic proteins was determined by gel-permeation chromatography and polarography. Data were examined statistically using ANOVA Student's 't' and Student-Newman-Keuls analyses. Significantly greater ($p=0.05$) concentrations of copper and zinc were found in hepatopancreas of reference (Hastings Arm) specimens; whereas gill tissues from Alice Arm specimens demonstrated significantly higher lead and molybdenum burdens. Metal-binding protein concentrations were uniformly high, regardless of site and ranged from 6.8 to 18.8 g kg^{-1}. Gel-permeation chromatograms of natural and ethanol-denatured hepatopancreatic cytosols suggest that the principal metal-binding component has a molecular mass of ca. 35 000 daltons. Analysis of chromatographic fractions for Cd, Cu and Zn indicate that Cd is the principal metal associated with the protein.

KEY WORDS: Lithodes, heavy metals, tissues, mine tailings, metal-binding proteins.

RESUME

On a évalué les charges de 32 éléments, par spectrométrie plasmatique à induction, des tissus branchiaux, musculaires et hépatopancréatiques du crabe doré (Lithodes aequispina Benedict) prélévés à deux sites à Alice Arm et à quatre sites à Hastings Arm, Colombie-Britannique. Les données relatives à six éléments: l'arsenic, le cadmiun, le cuivre, le plomb, le molybdène et le zinc ont été analysées en détail en raison de leur importance relativement à l'évacuation des déchets miniers de l'usine de molybdène AMAX à Alice Arm. On à également évalué la teneur en métalloprotéines des tissus hépatopancréatiques de chaque échantillon à l'aide de la polarographie impulsionnelle différentielle. La distribution du poids moléculaire des protéines cytosoliques a été déterminée par chromatographie sur gel et par polarographie. Les données ont été traitées statistiquement par analyse de variance, test de Student et test de Student-Newman-Keuls. On a décelé des concentrations significativement ($p=0,05$) plus importantes de cuivre et de zinc dans les tissus hépatopancréatiques des échantillons témoins (Hastings Arm), alors que les tissus branchiaux des échantillons de Alice Arm contenaient des charges significativement plus importantes de plomb et de molybdène. Les concentrations de métalloprotéines étaient uniformément fortes, quel que soit le site, et variaient de 6,8 à 18,8 g kg^{-1}. Les chromatogrammes sur gel des cytosols hépatopancréatiques naturels et dénaturés à l'éthanol laissent supposer que la principale composant métallique a une masse moléculaire d'environ 35 000 daltons. L'analyse des fractions chromatographiques de Cd, de Cu et de Zn indique que Cd est le principal métal lié à la protéins.

Mots-Clés: Lithodes, métaux lourds, tissus , déchets miniers, métallo-protéines

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INTRODUCTION

In 1981, Amax of Canada Ltd. began to discharge mine tailings from the Kitsault Molybdenum Mine directly to Alice Arm. The potential environmental impact of this discharge was assessed by a Scientific Review Panel (Burling et al. 1981) which concluded that increased loadings of cadmium, lead and zinc solids on Alice Arm sediments would result, with possible long-term impacts on benthic species. After a review of monitoring information obtained during the first nine months of operation, the Panel recommended that additional studies be undertaken to "explore the relations between heavy metals and Amax/Kitsault tailings and deposit feeders" (Burling et al., 1983).

Some heavy metals are especially toxic to certain marine organisms, particularly crustaceans (Brown and Ahsanullah, 1971; Reeve et al., 1976). However, marine organisms also are capable of developing tolerances to challenges by the heavy metals copper, zinc, cadmium or mercury through induction of metal binding proteins such as the metallothioneins (Olafson and Thompson, 1974; Klaverkamp et al., 1984). The inducible nature of metallothioneins and related proteins implies that they may play a role in the detoxification of the four metals mentioned above (and possibly others) and may therefore permit a quantitative assessment of 'stress' response in organisms to these pollutants.

The purpose of this study was to compare quantitatively trace metal and metal-binding protein concentrations in Golden King Crab (Lithodes aequispina Benedict) collected from Alice Arm, location of the AMAX/Kitsault mine tailings discharge, and Hastings Arm, a reference site for monitoring programs in the area. Golden King Crab are taken in commercial and native fisheries in the area and have been sampled and analysed as part of monitoring programs related to the tailings discharge.

Sloan (1985) points out that L. aequispina is a little known species biologically and has only in recent years become a focus of the commercial fishery in Northern B.C. coastal waters. Virtually nothing, aside from survey reports (Goyette and Christie, 1982a,b) has been published on the chemistry of this species nor, in fact, on the Lithodid family. This report represents the first comprehensive study of trace metals and metal-binding proteins in the species.

METHODS AND MATERIALS

Sample Collection

The study area for this investigation included Alice and Hastings Arms, two fjords which form the terminal arms of Observatory Inlet in northern British Columbia (Figure 1).

Golden King Crab (Lithodes aequispina Benedict) were collected in commercial King Crab pots during the period October 27 to November 2, 1983. Pot dimensions were 1.8 x 1.8 x 0.8 m with a mesh size 9 x 12 cm.) The pots were baited with frozen herring suspended in perforated plastic containers. The soak times of the sets varied from station to station and ranged from 23.3 to 45.2 hours.

As there is a high incidence of the parasite Briarosaccus callosus in L. aequispina within the study area (Sloan, 1984), only unparasitised crabs (as determined by the lack of the parasitic 'sac' on abdomens) were sampled to eliminate this variable. Six adult Golden King Crab (3 male, 3 female) were collected for metal-binding protein and heavy metal determination from each of three locations; upper Alice Arm, (Group 1, near the outfall), mid-Alice Arm (Group 2) and Hastings Arm (Group 3). Specific collection data included carapace length, carapace width, wet weight, sex and reproduction stage for each crab (Table 1). Sampling locations are illustrated in Figure 2. Specific pot depth for each location is also presented in Table 1.

Sample Preparation

All dissections were conducted within two hours of specimen collection. Crabs were dissected on a bed of crushed ice in a stainless steel laminar-flow hood. Precleaned (acid washed, deionized water rinsed) disposable polyethylene knives and spoons and polyethylene gloves were used for tissue dissection and handling. Separate instruments were used for each tissue type, and were changed for each crab. Precleaned stainless steel scissors were used to cut the crab carapace. Replicate samples of hepatopancreas, gill and muscle tissue for protein determinations were removed from each crab, placed in individual 'Whirlpak' bags and weighed (wet weight 1.6 - 11.1 g). Each sample was then covered with 3 mL of buffer solution (50 mM TRIS, 10 mM mercaptoethanol, 100 μM phenylmethylsulfonyl fluoride (PMSF) and 0.02% NaN_3 at pH 8.6) and immediately frozen on dry ice.

Similarly, replicate samples of hepatopancreas, gill and muscle tissue (wet weight > 5.0 g) were removed from all crabs and treated as previously described (excluding addition of TRIS buffer) for analyses for trace metals.

All samples were shipped on dry ice in plastic coolers. Upon receipt in the lab, samples were immediately transferred to a standard chest freezer where they were held until analysis.

In the laboratory, tissue for protein assay was thawed and homogenized in approximately nine volumes of TRIS-buffer solution used

in the preservation. The tissue and buffer were homogenized (2 min; Brinkmann Polytron) and an aliquot from each sample, approximately 25 mL, was centrifuged at 15 000 rpm (27 140 x g) for 1 h at 5° C.

After centrifugation, the supernatant beneath a lipid emulsion surface layer was partially removed by syringe and passed successively through a GF/C prefilter (Whatman) and a 0.2 um membrane filter (Millipore HA).

For each sample, aliquots of filtered cytosol were analyzed for total thiolic protein (see below). In addition, 4.0 g of filtered cytosol from each sample was transferred to a 10 mL centrifuge tube and 4.0 g absolute ethanol was added. These samples were aged overnight before being centrifuged at 15 000 rpm (27 140 x g) for 45 min at 0-5° C to remove precipitated high molecular-weight proteins. The supernatant solution was then removed and analysed for thiolic proteins. Samples that were not analyzed immediately were stored at 4° C for a maximum of 48 h before analysis.

Duplicate 1.5 g aliquots of original sample homogenates were removed to determine tissue dry weights. Aliquots were accurately weighed into tared disposable weighing boats, lyophilized and reweighed. For every sample pair a blank of buffer solution was lyophilized to provide a corrected weight of each sample aliquot.

Analytical Methods

Protein

Details for the protein determination are described by Thompson and Cosson (1984) and by Olafson and Sim (1979). The differential pulse polarographic (DPP) procedure was improved further by lowering cell temperature to ca. 5° and modifying the formulation of the Brdička buffer (Thompson et al., unpublished). Detection limits, employing the International Union of Pure and Applied Chemistry (IUPAC) criterion of 3 S_B/m (where S_B is one standard deviation of the analytical blank and m is the slope of the calibration line) ranged 1 to 4 ug L^{-1} of cytosol depending upon daily operating conditions.

Standard calibration curves were generated using purified metallothionein of the crab, Scylla serrata*, dissolved in 'TRIS' buffer.

Separation of cytosolic protein components was accomplished using standard gel permeation chromatographic techniques. The jacketed 26 mm x 100 cm column consisted of Sephadex G-75 equilibrated in 10 mM TRIS-HCl buffer (pH 8.6) containing 2 mM mercaptoethanol and 0.02% NaN_3. Separations were performed at 0-5° C on an LKB Ultrarack II fractionator. Absorbance and transmittance of components were monitored at 254 nm. Flow rates were controlled by peristaltic pump. Five mL fractions were collected at 0.69 mL min^{-1}.

Fractions were assayed for thiolic protein and trace metal (Zn, Cd, Cu) content. The latter was accomplished using a Perkin-Elmer Model

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* The S. serrata protein was a gift from Dr. R.W. Olafson, Univ. Victoria.

503 G atomic absorption spectrometer fitted with a Model 400 Heated Graphite Analyser. Deuterium continuum background correction was employed and absorbances were recorded on a strip-chart recorder.

All calibrations and calculations of unknowns were made utilising a polynomial curve fitting computer program.

Lipids

Lipid extractions of 15 crude hepatopancreas homogenates were performed using the methanol-chloroform procedure of Bligh and Dyer (1959).

Metal Determinations

All utensils employed in trace-metal determinations were scrupulously acid-washed and kept under 'double' Milli-Q (Millipore) deionized water or quartz-distilled water prior to use. Quartz digestion dishes were air-dried in a Class 100 clean-room and were pre-tared.

Frozen samples in plastic bags were removed to the clean-room where they were partially thawed and then aportioned into the quartz dishes using stainless-steel scalpel blades. Blades were held with gloved fingers to avoid contamination by scalpel handle (Patterson & Settle, 1976).

The dishes, with tissue, were weighed and placed in covered, clean polyethylene storage trays. Trays with samples were taken from the clean room to the freezer and refrozen in preparation for freeze-drying. Frozen samples were placed in the quartz chambers of a low temperature ashing instrument (LTA; International Plasma Corp.) and freeze-dried overnight. Tissue samples were reweighed to obtain the dry weight and then immediately returned to the LTA and ashed for 7 h at an oxygen flow rate of 700 cc min^{-1} and maximum forward power of 160 watts. After cooling, sample were reweighed to obtain ash weights and returned to the clean room where they were wetted with 1.0 mL quartz-distilled (QD) nitric acid. They were covered and allowed to stand at room temperature for several hours. Three samples at a time were removed from the tray and 2.0 mL perchloric acid (Ultrex, J.T. Baker) was added. The mixtures were then taken to near-dryness. Samples were quantitatively transferred with QD water (approx. 20 mL) to acid washed, QD water-rinsed polyethylene bottles using plastic transfer pipets and the weight of solution accurately determined. Samples were acidified with 200 ul QD HCl and stored.

Analysis was performed by inductively coupled plasma spectrometry (ICPS). The instrument provided an analytical capability for 18 trace elements and 14 minor elements:

	Trace Elements		Minor Elements	
*	Arsenic	(As)	Aluminum	(Al)
	Boron	(B)	Barium	(Ba)
	Beryllium	(Be)	Calcium	(Ca)
	Bismuth	(Bi)	Iron	(Fe)
*	Cadmium	(Cd)	Potassium	(K)
	Cobalt	(Co)	Lithium	(Li)
	Chromium	(Cr)	Magnesium	(Mg)
*	Copper	(Cu)	Manganese	(Mn)
	Mercury	(Hg)	Sodium	(Na)
*	Molybdenum	(Mo)	Phosphorus	(P)
	Nickel	(Ni)	Silicon	(Si)
*	Lead	(Pb)	Strontium	(Sr)
	Antimony	(Sb)	Titanium	(Ti)
	Selenium	(Se)	Zirconium	(Zr)
	Thorium	(Th)		
	Uranium	(U)		
	Vanadium	(V)		
*	Zinc	(Zn)		

Of the 18 trace elements only six (*) where were identified as most pertinent to the mine discharge and of interest in the current study. The elements arsenic, cadmium, copper, molybdenum, lead, and zinc will be discussed in detail. Data for the remaining trace elements and all minor elements are available upon request.

Because lead data by ICPS were below or near the detection limit in most cases, samples were analysed further using the heated graphite analyzer and A.A. spectrometer described earlier for protein analysis.

ICPS analysis was performed by Quanta Trace Labs, Burnaby, B.C.

Blanks: A total of 15 reagent blanks which were prepared with each batch of tissue digests was analysed together with the digests.

Reference Materials

As a means of determining the accuracy and precision of our methods eight to eleven replicates of each of three biological reference materials were included in the study. The three reference materials used were:

TORT-1, Lobster Hepatopancreas	(NRC Canada)
SRM 1566, Oyster	(NBS Washington)
SRM 1577, Bovine Liver	(NBS Washington)

These freeze-dried tissues were subjected to the same treatment as crab tissues from the ashing step on. Certified values and our values for trace metals in these materials are shown in Table 14.

Limits of Detection (LOD)

The generation of LOD values for the six elements is discussed in the Appendix.

Electron Microscopy

To explore the possible source(s) of lead in the Alice Arm gill samples, an initial examination of tissues from some of the 18 crabs collected for the study was undertaken. Since a possible source of the lead was particulates occluded in gill lamellae, particulates were identified using scanning electron microscopy (SEM). Subsequent determination of the elemental composition of the particulates was accomplished by energy dispersive X-ray irradiation (EDX) using a JEOL Model JEM-1200X system.

Statistical Analysis

Unpaired data for As, Cd, Cu, Mo and Zn were examined for outlying observations using Chauvenet's criterion. Upon removal of an outlying observation for a given group, means and standard deviations were recalculated and the set retested for outliers. Statistical tables outline the results of the Analysis of Variance with Student-Newman-Keuls (SNK) tests for site (Alice Arm outfall, Alice Arm mid-channel and Hastings Arm), tissue (gills, hepatopancreas and muscle) and the site by tissue interaction. Specifically, the model used was:

$$Y' = u + \text{tissue} + \text{site} + \text{duplicates} + (\text{tissue} \times \text{site}) + \text{error}$$

Tables 2 through 12 describe the results of the analyses. When a factor (ie. tissue, site, etc.) was found to be significant, in this case at the 5% level ($p \leq 0.05$), an SNK test was performed to locate the source of the difference. This is reported in the tables through the use of letters, such that means within a factor followed by the same letter do not differ significantly.

Statistical analysis of the lead data from flameless atomic absorption spectrometry was performed in a different manner. In this case single-tailed Student 't' tests were made to determine if means were statistically different from calculated detection limits (see Appendix) for this element. Thus, for example, means for gill tissues from the two Alice Arm groups were compared to detection limit of 0.30 mg kg^{-1}.

RESULTS

Trace Elements

Arsenic

Data and statistical analysis for arsenic in each tissue type of L. aequispina are shown in Tables 2 and 3. These data indicate that gill and hepatopancreatic tissues contain statistically similar amounts of

the element. Significantly greater ($p \leq 0.05$) quantities were found in muscle tissue.

No significant differences were observed among the three sites in any of three tissue types (Fig. 3).

Cadmium

Tables 4 and 5 show data for cadmium. Here also, no significant differences were found for any tissue type among the three groups although there was an apparent trend to higher concentrations in gills from both Alice Arm groups. Muscle tissue accumulated more than two orders of magnitude less Cd than gills or hepatopancreas. Over all sites, differences in accumulated amounts were significant for all tissue types, with gills attaining the greatest quantities. Differences in the three tissue types are illustrated in Figure 4.

Copper

Concentrations of copper (Tables 6 and 7) ranged from an overall mean of 37 mg kg^{-1} in muscle tissues to 493 mg kg^{-1} in the hepatopancreas. Comparison of each tissue with location indicates that no significant differences or trends exist in gills whereas concentrations were significantly higher in hepatopancreas and muscle from Hastings Arm samples (Fig. 5). This observation extends also to overall tissue values (Table 7). Overall site data show that the hepatopancreas is a major site of accumulation for copper.

Molybdenum

Molybdenum concentrations (Tables 8 and 9) were low in all three tissue compartments and values for muscle were close to the calculated detection limit (Appendix). However, statistically significant differences were found, notably for mid-Alice Arm (Group 2) gills which contained greater quantities than those from Group 1 or Group 3. Differences were also found for overall sites, indicating that gills tended to concentrate greatest quantities, and for overall tissues which also indicated that Group 2 specimens had accummulated significantly greater amounts of the element (see Fig. 6).

Lead

Lead concentrations reported in Table 10, were redeterminations using flameless atomic absorption spectrometry because ICPS results were near or below instrument LOD. Even with the more sensitive technique, the element was undetectable in all muscle samples (LOD: 0.15 mg kg^{-1}) and in gills and hepatopancreas from Group 3 (Hastings Arm) (LOD: 0.3 and 0.2 mg kg^{-1} respectively). Accordingly, simplified statistics (Student's 't') were used to ascertain whether or not means obtained for lead in gill and hepatopancreas from the two Alice Arm groups were significantly greater than calculated tissue LODs. The Group 1 mean of 1.15 ± 0.89 mg kg^{-1} for gill tissue was found to be significantly greater than the LOD at $p<0.05$. The Group 2 mean of 1.59 ± 0.67 mg kg^{-1}

was significant at $p<0.005$, in spite of high relative standard deviations for the two data sets.

Hepatopancreas lead burdens in Groups 1 and 2 samples were measureable (Fig. 7). Means were at or marginally above the LOD of 0.1 mg kg^{-1} and neither was statistically greater than that value. Overall, lead burdens were considerably greater in Alice Arm specimens of L. aequispina than in those from Hastings Arm.

Zinc

Data shown in Tables 11 and 12 for zinc indicate the hepatopancreas is the preferred site of accummulation. Overall site statistics show significant differences among tissue types. Comparable burdens of zinc were found in overall tissues for the two Alice Arm groups. Significantly greater quantities were found in Hastings Arm samples which is reflected predominantly in the hepatopancreas where the mean (150 mg kg^{-1}) was greater than either group 1 or 2. Neither gills nor muscle data indicated significant differences or trends. Relative tissue concentrations are shown in Figure 8.

Metal-binding Proteins

Pulse polarographic analysis for metal-binding proteins (MBP) was performed on the ethanol-denatured cytosolic component of 18 hepatopancreas samples from L. aequispina. Table 13 indicates that concentrations of MBP varied over a range of 6.8 to 18.8 g kg^{-1} of dry tissue. Means for Groups 1, 2 and 3 are almost identical, the range being only 0.6 g kg^{-1}.

To further determine the nature of the protein giving rise to the polarographic response, samples of denatured and non-denatured cytosols were chromatographed on Sephadex G-75 gel. The column had been previously calibrated with standards of known molecular mass so that we were able to assess the mass distribution of the cytosolic components from the hepatopancreas. The chromatographic profiles are illustrated in Figures 9 and 10. Figure 9 shows the distribution of Cd, Cu and Zn and polarographically active protein before ethanol denaturation. Thiolic protein was detected primarily in a mid-to-high-molecular weight range with a maximum at 3.4×10^4 daltons. A small peak was observed also in the range of 1.0 to 1.2 x 10^4 daltons. Cadmium, copper and zinc were all highly associated with the void volume fractions ($\geq 6.0 \times 10^4$ daltons). Cadmium exhibited additional peaks coincident with the mid-range protein peak at 3.0 to 3.5 x 10^4 daltons and in the very low-molecular weight range ($<1.0 \times 10^4$ daltons). Copper was found in fractions associated with a molecular weight range of 2.0 to 2.5 x 10^4 daltons and also in the very low range $<1.0 \times 10^4$ daltons, however, these did not coincide with thiolic protein activity. Strong zinc peaks were measured at 2.5×10^4 daltons and in the very low range. These, also, were not coincident with thiolic protein.

The purpose of denaturation is to remove thiol-containing materials, which are not metal-binding proteins. Denaturation is generally performed for our purposes by heating cytosol to 85° for 5

minutes or by addition of an equivalent volume of alchohol. Metal-binding proteins are inherently heat-stable and are stable in a 1:1 ethanol solution. Figure 10 illustrates the result of gel-permeation chromatography of ethanol-denatured cytosol from L. aequispina hepatopancreas. While metal concentrations were reduced in the void-volume fractions there are still quantities of Cu and Zn and considerably less Cd. The Zn peak observed at 3.0×10^4 daltons was reduced substantially while Cd in the same range increased 4-fold. The strong Cd peak is associated with the main protein peak which was reduced only marginally by denaturation. A low response in the 1.0 to 1.2×10^4 daltons range also was unaltered. Strong metal responses in the $<1.0 \times 10^4$ daltons fractions remained also. A copper response at ca. 2.0×10^4 in the non-denatured sample was reduced to below back-ground levels after denaturation.

Lipid content was determined by an ethanol-chloroform extraction of duplicate samples of 15 available samples of hepatopancreas (Table 13). Concentrations ranged from ca. 11 to ca. 45 percent (dry wt. basis) and correlated significantly to polarographically active protein content in an exponential relationship (log MBP = 0.429 - 0.0137 [lipid]; r = -0.94) although linear and log-log models were also highly correlated (r = -0.91 in both cases).

DISCUSSION

Reference Materials

An important consideration in any exercise of this kind is the quality of data obtained from analysis of the various tissue types. Within the last half-decade a number of biological reference materials have been developed by the National Research Coucil of Canada and the National Bureau of Standards in Washington, D.C. Various types of tissues were analyzed exhaustively in an effort to establish certified values for a number of minor and trace elements. In this study eight to eleven samples each of freeze-dried lobster hepatopancreas (TORT 1, NRC), oyster tissue, (SRM 1566, NBS) and bovine liver, (SRM 1577, NBS) were subjected to the same digestion and analytical procedures used for the crab tissues. Analytical data and the respective certified values are shown in Table 14 for the elements discussed in this report.

There is good agreement between our data and the certified values for all elements in NBC bovine liver and oyster materials and for all but copper and lead in the lobster hepatopancreas, for which recovery was low. These assurances suggest that the large standard deviations for elements, such as copper, in this study are more likely attributable to natural variation rather than analytical error.

Trace Elements

Arsenic

Data for As in all tissues from L. aequispina demonstrate the natural propensity for crustaceans to accummulate this element, especially in muscle tissue. It is not uncommon to determine quantities greater than 100 mg kg^{-1} (dry wt.) in crabs in uncontaminated waters (Goyette and Christie, 1982a,b).

Data reported here (Tables 2 and 3) agree well with those obtained in previous analyses of L. aequispina muscle tissues (Farrell and Nassichuk, 1984).

Cadmium

Cadmium (Tables 4 and 5) is stored preferentially in gill and hepatopancreatic tissues which are not commonly human food sources. Concentrations of Cd in edible muscle were over two orders of magnitude lower than in the two major accumulation sites. It is probable that Cd is held in gills and hepatopancreas by metal-binding proteins (MBP) for which evidence in this study will be discussed below. In gills MBP would be induced by dissolved Cd, while nutritional sources would induce production in hepatopancreas. Water column studies in Alice Arm during the period of discharge from the AMAX mill (prior to November, 1982) indicated near-bottom Cd-enrichment in both the dissolved and particulate phases when compared to stations outside the inlet (Wong and Stukas, unpublished). Dissolved Cd values approached 200 ng kg^{-1}. Although there was an observed trend to higher Cd in gills from Groups 1 and 2, differences were not statistically significant. Nothing is currently known about clearance rates for Cd in Lithodid crabs.

Copper

Unlike cadmium, lead, zinc, or molybdenum, copper is not found in the tailings in quantities above those in natural sediments from the same area (Burling et al. 1981; Thompson and Paton, unpublished manuscript). Likewise, increased amounts of this element are not found in pore waters from deposited tailings suggesting that exposures to this element within Alice Arm would also be normal (Thompson and Paton, unpublished).

Our results indicate that, of the three tissues studied, the hepatopancreas contained by far the greatest quantity of copper. Copper is an important element involved in oxygen transport (haemocyanin) and the hepatopancreas is the primary site for processing of the haemolymph.

Gills also contain appreciable quantities of this element, because of their role in oxygen transport.

The salient features of the results for copper are the significantly greater quantities found in muscle ($p<0.001$) and hepatopancreas ($p<0.05$) samples from Hastings Arm. Keeping the small sample size in mind, a number of possible and plausible reasons can be invoked to explain these increases. Recent studies by Thompson et al. (unpublished) have shown that the extractability of copper from tailings in 1 M HCl is less than it is from natural sediments. This difference in extractability can be used as an operational measure of the relative biological availability of elements to interacting organisms (Luoma and Jenne, 1976; Luoma and Bryan, 1981). For example, compared to extraction in aqua regia ('total' recovery) submerged tailings from a station near the outfall yielded only 3-4% of the 'total' after leaching in the milder conditions of 1 M HCl. Natural sediments at three stations in Hastings Arm and one in Portland Inlet, in comparison, yielded 43-60% of the 'total' under the same conditions. Within Alice Arm percentages of the 'available' copper increased up-and-down-inlet from the outfall as dilution by natural sediments increased. Aside from the differences in relative availability of copper it should be noted that absolute concentrations are higher in Hastings Arm sediments (Thompson et al. unpublished).

While it does not seem likely that depleted copper concentrations in crab tissues would be a result of direct interactions between organism and sediment, they may represent reduction of availability of the element through the food chain.

Alternatively, higher concentrations in Hastings Arm samples may suggest a source of copper other than natural sediments. The slag and tailings at the abandoned copper mine at Anyox (Figure 2) is a potentially significant source of copper and other elements (Thompson and Paton, unpublished; Goyette and Christie, 1982a). Aside from the expansive distribution of material already on the inlet floor (Granby Bay) there is a continuous supply of dissolved metal due to acid leaching and runoff from shoreline deposits. Operations in Granby Bay began in 1912 and ceased in 1935. Because of the long existence of this source, its influence might be felt throughout the food web over a large portion of Hastings Arm.

Differences in copper concentrations in tissues in crabs from the Hastings and Alice Arms would also lend support to the hypothesis that little or no migration over the sill exists and that the crab populations in the two inlets are isolated from one another (Sloan, 1985).

Molybdenum

Very little is known about the interaction of molybdenum with marine biota. It is not considered to be environmentally toxic and is not an internationally regulated substance; although the discharge permit for the mine does specify a maximum of 1 mg L^{-1} in solution.

In spite of considerable tailings enrichment by this element there is no indication of Mo bioaccumulation in muscle and hepatopancreas, whereas there is a slight but significant ($p \leq 0.05$) increase in gill tissue from Alice Arm (Table 9). Gill concentrations may reflect exposure either to fine Mo-bearing particulates in the tailings zone of Alice Arm or to bottom waters enriched in dissolved Mo. Losher (1985) has reported Mo-enriched pore-water in deposited tailings collected in October, 1983.

In studies of copper/molybdenum mine tailings in Rupert Inlet, B.C. Pedersen (1985) has shown a marked difference between natural and mine-derived sediment porewater Mo concentrations. In natural sediments (Holberg Inlet) Mo fluxes are into the sediment. In areas of tailing deposition, porewater concentrations of this element increased by as much as 10^3 and flux reversed.

Lead

Acquisition of meaningful lead concentrations in environmental samples has long been an 'El Dorado' for chemists who have had to contend with contamination of samples through all stages of sampling and analysis. Realistic values for this element in sea water have been attained only in recent years by careful control of the sampling process and the use of clean-room methodology. For biological samples the problem of contamination has also existed, although concentration ranges are probably 3-or more orders of magnitude greater (Settle and Patterson, 1980). It is only through the use of rigorously controlled sampling and work-up methods that we have been able to acquire crab lead data in which we have a degree of confidence.

In this study, we have found that lead was detectable only in gill and hepatopancreas samples from Lithodes specimens collected from Alice Arm. Lead in Hastings Arm samples was non-detectable in all cases. The data for gills shown in Table 10, although highly variant, demonstrate significant uptake of this element. Uptake in this case could be either via dissolved or particulate phases. Detectable but statistically insignificant quantities of lead in hepatopancreas tissues may represent uptake from food, or possibly transport via haemolymph from the gills. Regardless of the source, it is apparent that lead remains bound essentially to gills, with little or no transport to hepatopancreas or muscle. It is possible that another major sink (eg. carapace) could also exist.

Studies of the waters of Alice Arm and Observatory Inlet during the discharge period (Wong and Stukas, unpublished manuscript) have shown that like cadmium, lead increased markedly in both dissolved and particulate phases. At one station below the outfall dissolved Pb was 112 ng kg^{-1} at 350 m (near bottom). In contrast, lead in bottom water from Observatory Inlet was 0.32 ng kg^{-1}. Isotopic analysis of these samples indicated that the dissolved Pb in Alice Arm was derived essentially from the mine waste.

Although data would suggest that lead in gills had been removed from solution there was a possibility that fine particulates occluded in

the tissues could be responsible for the observed high concentrations. To clarify the physico-chemical status of lead and other elements in gills we undertook a brief examination of a selection of samples by scanning electron microscopy (SEM) and energy dispersive x-ray analysis (EDX). This technique permits elemental analysis of individual μm-sized particles embedded in tissues. While observed particles were numerous, none contained lead, zinc, cadmium or molybdenum. Elemental composition was characteristic of naturally occurring, siliceous minerals. We have since undertaken further studies using alternative tissue preparation techniques which may provide further information. However, current information would suggest that the elements under investigation are chemically bound in gill tissues.

Zinc

Like copper, zinc plays an important role in the biochemistry of most organisms. A number of enzyme functions (eg. carbonic anhydrase) depend upon this element. Because zinc is an essential element, an organism's tolerance for anomalous exposures is probably greater than that for an element (eg. lead) that has no physiological function. Zinc homeostasis is now thought to be partially controlled by the metal-binding protein, metallothionein, which is induced by exposure to zinc, cadmium or copper and perhaps a few other elemments (eg. Olafson and Thompson, 1974; vide infra).

In this study, hepatopancreatic tissues contained the greatest proportion of this element (Table 12) with lesser amounts in muscle and least in the gills. Average concentrations of 371 mg kg^{-1} in the hepatopancreas reflect, most likely, the physiological importance of this element. Insignificant differences in means for gill and muscle tissues were found among the groups. However, similar to copper, we determined that significant ($p<0.05$) differences for zinc existed between group 3 and groups 1 and 2. Overall tissue concentrations of Zn were significantly greater in Hastings Arms samples. This result again suggests that zinc is more available and/or more plentiful in Hastings Arm sediments than it is in those of Alice Arm. An examination of data for the relative availability of this element from the two sources (Thompson et al. unpublished) indicates a situation parallel to that for copper. Ratios for zinc recovered by the HCl and aqua regia leaches were lowest (2%) for tailings derived material and highest (50-60%) for natural sediments near and outside the sill in Alice Arm. Because of this difference and even though absolute Zn concentrations may be greater in tailings, the amounts actually available to the food chain in Alice Arm may be appreciably less. Again, the slag at Granby Bay cannot be ruled out as a source, both by submarine leaching and by terrestrial acid drainage. The potential for this site as a major source of Zn and Cu contamination cannot be overlooked and is currently under further investigation.

METAL-BINDING PROTEINS

About 25 years ago a protein isolated from equine renal cortex was found to contain cadmium, zinc and copper (Margoshes and Vallee, 1957). As well, the protein possessed a high ℓ-cysteinyl residue and had a molecular weight of about 10 000 daltons. It was later found that this protein was induced in mammals by challenges with any one of the three metals and that initial treatment made an animal resistant to high, normally toxic doses. The protein, metallothionein, has been implicated in a detoxification mechanism whereby an organism generates the protein as a means of preventing interaction of critical enzyme functions with the metals (see Kägi and Nordberg, 1979 for a review of the subject).

Olafson and Thompson (1974) reported the first identification and induction of metallothionein - like proteins in marine mammals and teleosts. Since that time it has been established that these proteins, which vary somewhat in structure and metal content, depending on the organisms, are ubiquitous in the biosphere. It has also become clear that in addition to their role as a detoxifying medium, these proteins may be instrumental in homeostasis of zinc and copper (Cherian and Goyer, 1978).

In recent years a great deal of effort has been placed upon the quantification of these proteins in order to establish such factors as dose-response relationships, induction kinetics and ambient concentrations of the proteins in various species. One of the most promising quantification methods involves the use of differential pulse polarography in Brdička's electrolyte (Pelaček and Pechan, 1971; Olafson and Sim, 1979; Thompson and Cosson, 1984). This technique requires only milligrams of sample from which microliters of cytosol are used for the assay. Currently 1-4 $\mu g\ L^{-1}$ of the protein in solution are detectable (Thompson and Cosson, 1984; Thompson et al., unpublished).

The occurrence of metal-binding proteins (MBP; termed thus, as it may be that non-metallothionein proteins are involved) in marine invertebrate species has been studied only in the past few years, and, accordingly, little is known about concentrations and structure. In fact, it is apparent from recent studies (Thompson et al., unpublished; Engel and Brouwer, 1981; Siewicki et al., 1983; Ridlington & Fowler, 1979) that there may be a complex mixture of these proteins in shellfish tissues such as the oyster digestive gland.

An important part of our study of metal distribution in L. sequispina was to endeavor to determine concentrations of metal-binding proteins in tissues of this species. Since this was the first time, to our knowledge, that quantification of these proteins had been attempted there were no guidelines as to concentration of the protein and the characteristics of the tissues upon work-up.

Hepatopancreas was chosen as the study tissue because of its known, important metal cycling function in crabs. Results of the duplicate analysis of the 18 samples are shown in Table 13.

After ethanol denaturation there was, for most of the samples, only a slight drop in polarographic response, indicating that the thiolic proteins were essentially metal-binding species. Concentrations in the range of 7 to 19 g kg^{-1} are the highest we have measured in our laboratory to date in any species. Previous studies with the Pacific Oyster, Crassostrea gigas showed digestive gland MBP in order of 5 g kg^{-1} dry weight (Imber et al., unpublished) and concentrations in the hundreds of mg kg^{-1} have been reported in fish livers (Roch et al., 1982).

From the data in Table 13 it is quite apparent that there are no statistical differences in MBP concentrations among the three sample groups. It would follow that there is no correlation between the metal concentrations (Tables 2-12) and the protein concentrations.

A complicating factor in our interpretation of the protein data was their inverse, non-linear relationship with the lipid content of the hepatopancreas (Table 13). The nature of this relationship remains to be determined. It is possible that the protein is lipid-soluble, in which case when the lipid content is high, a greater amount of the protein is associated with it. It may be, instead, that protein and lipid production are competitive processes which relate to seasonal changes or to the reproductive cycle. Comparison of the sex of each specimen, with lipid content, however showed no relationship in this study. Further studies to determine if the protein is lipid-soluble will be required.

Further characterization of the cytosolic proteins in Lithodes hepatopancreas was accomplished by G-75 gel-permeation chromatography. Analysis of fractions collected from native samples (Figure 9) indicated that the major polarographically active protein had a molecular mass of 3.0×10^4 to 3.5×10^4 daltons. This is about three times the mass of metallothionein determined by gel permeation techniques. Only a small peak in this lower molecular mass range was observed. The only metal showing any degree of association with the major protein peak was cadmium. Cadmium also appeared in high concentrations in void volume ($\geq$ 60 000 daltons) fractions and in fractions at or below 10 000 daltons. Zinc and copper were identified in void volume fractions and in mid-and lower-weight fractions. In neither case was there any strong correlation with thiolic protein.

After ethanol denaturation cadmium associated with the thiolic protein peak increased four-fold (Figure 10) while decreasing in the void volume fractions. At the same time protein response decreased by one-third. This suggests that some redistribution of cadmium from the high molecular mass range occurred. It is possible that additional coordination sites on the protein were occupied by Cd made available in the denaturation step. Denaturation resulted in nearly total loss of zinc in the 20 000 to 25 000 daltons range although some remained associated with the Cd and protein peaks. This would suggest that the zinc binding protein moiety may have been essentially enzymatic.

Olafson et al. (1979a,b) reported isolation and characterization of a metal-binding protein induced by Cd in hepatopancreas of the swimming

crab Scylla serrata. Molecular weight (by GPC), amino acid analysis, metal content and absorbance ratios at 250 and 280 nm identified the protein as metallothionein. A limited number of other studies have suggested that differing crab species produce proteins with varying characteristics. Rainbow and Scott (1979) reported two metal-binding proteins in hepatopancreas of Carcinus maenas of 27 000 and 11 500 daltons where the lower mass moiety contained the greater quantities of Cu, Zn and Cd.

Two heat-stable proteins of 10 600 and 9 400 daltons were reported in hepatopancreas of the blue crab Callinectes sapidus by Wiedow et al. (1982). Only one Cd-BP of 13 000 daltons was determined in the same species by Engel and Brouwer (1984).

The major metal-binding thiolic protein found in this study is 8 000 daltons higher in molecular mass than that reported by Rainbow and Scott (1979), which it resembles most closely. Without further purification and characterization by amino acid analysis and sequencing it is not possible to make any further conclusions regarding its relationship to metallothionein. That the relative molecular mass is 3-fold that of MT it might be assumed that the Lithodes protein may be a trimeric aggregate of MT. However, we suspect this not to be the case, as polymerization is generally the result of oxidation of -SH to -S-S- linkages. Oxidation is generally prevented by use of 2-mercaptoethanol (Overnell, 1984; Minkel et al., 1980) which prohibits formation of disulfide linkages. Mercaptoethanol (2 mM) was employed at all stages of homogenization and chromatography in this work. Although there is a possibility that a higher concentration of 2-mercaptoethanol could be required there is no evidence to suggest this is the case nor is there any evidence in the literature to warrant higher concentrations of this reductant.

CONCLUSIONS

We have reported six trace metal burdens in three tissue types for the Golden King Crab, Lithodes aequispina. Statistically significant differences between groups from Alice and Hastings Arms were found for copper and zinc in hepatopancreas and molybdenum and lead in gill tissues. For copper and zinc these differences reflected higher tissue burdens of these elements in reference (non-perturbed) animals which suggests that: 1. A significant source (such as the abandoned smelter at Anyox) exists in Hastings Arm; or 2. These elements in mine tailings in Alice Arm are biologically less available to the species than they are in natural sediments.

Conversely, for molybdenum and lead, elevated gill tissue burdens in Alice Arm samples (Groups 1 and 2) indicate that a source of these elements may be the deposited tailings on the floor of the inlet. Studies of gill tissue samples by electron microscopy and energy dispersive x-ray analysis suggest that these elements are chemically bound rather than occluded in the particulate phase.

Metal-binding protein concentrations in hepatopancreatic tissues were high in all samples, regardless of sampling site, and no

differences between groups were observed. Further studies will be needed to elucidate the relationships between MBP and lipid content.

The major binding protein determined by gel-permeation chromatography had a molecular mass of 35 000 daltons. Cadmium was the dominant associated metal. Additional studies would be required to ascertain the properties of this protein and its relationship to metallothionein-like compounds.

It is recommended that a larger follow-up sampling of L. aequispina be made to establish a better data base for this species in the waters of Alice Arm and the adjoining inlets.

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APPENDIX

ESTIMATED LIMITS OF DETECTION (LOD) FOR TRACE ELEMENTS IN TISSUES OF LITHODES AEQUISPINA

The currently acceptable definition of the limits of detection for an element is based upon a calculation of the standard deviation of the signals obtained from a series of blank deteminations for the method being employed. Earlier calculations were often based upon a value calculated for the peak-to-peak noise of the instrumental response, a value which generally underestimated LOD.

The inductively coupled plasma spectrometric technique (ICPS) employed in this work is such that blank analysis usually renders a 'less-than' figure as it did in all but one of the 32 elemental assays in this work and all of the six discussed in detail. A more rigorous calculation of LOD using the concept of propagation of error (Long and Winefordner, 1983) requires values of the slope and intercept of the calibration curve. These data were also not available from the ICPS instrumentation because of characteristics of the computer software.

Table 15 provides calculations of estimates of the method LOD for each element and each tissue type discussed in the foregoing report. The calculations employ the instrument LOD (i.e. the ICP "less than" value for the blank solutions) and values for each element derived from data from one or more of the standard reference materials (see Table 2). Thus these best estimates will reflect a range within which the method LOD might be expected to lie.

LOD values were calculated as follows:

a. From blank data,

$$LOD = 3 \left[\frac{B \times 0.020 \times RSD}{W \times 100} \right] = 3S \ (mg\ kg^{-1})$$

where B is the reported 'less-than' value for respective blank in mg L^{-1} of solution.

RSD = a 'worst case' value of the relative standard deviation of an element obtained from reference material data.

W = nominal weight of tissue used (dissolve in 0.020 L)

S = an estimate of the standard deviation

b. From reference material data,

$$LOD = 3 \left[\frac{S_{sol} \times 0.020}{W} \right] (mg\ kg^{-1})$$

where S_{sol} = standard deviation (in mg L^{-1}) of the elemental concentration in 0.020 L of the digest solution and W = nominal weight (kg) of tissue (as above).

Of the data presented in Table 15 only the range for Zn appears to be excessively broad. This can be accounted for in the relatively high quantities found in all three of the reference materials. More desireable would have been a value closer to the LOD, perhaps obtained using a set of samples spiked at detectable concentrations near the LOD. Of the data shown in Tables 2-12 only those for Mo in muscle and Zn in gills would be considered to be at or near the LOD for that particular tissue.

c. Detection limits for lead by graphite furnace AA,

LOD for lead determined by graphite furnace atomic absorption was calculated for each tissue using data obtained from 8 replicate analyses of the SRM 1566 (oyster). The mean and standard deviation for lead were 0.29 and 0.05 mg kg^{-1} respectively. The certified lead content is 0.34 mg kg^{-1}. Using the figure 0.05 for S_B we calculated the LOD for 1 g samples to be 3 S_B or 0.15 mg kg^{-1}. For 0.5 and 1.5 g samples the LOD was 0.30 and 0.10 mg kg^{-1} respectively.

Table 1. Golden King Crab Collection Data - Alice and Hastings Arms, October 27 - November 3, 1983.

Location	Sample #	Carapace Length (mm)	Carapace Width (mm)	Whole Wet Wt(Kg)	Males	Females	Female With Eggs	Female With Matted Setae	Pot Depth Meters
Alice Arm									
	LA6	141	147	1.45		X	NO	YES	329
	LA7	139	145	1.65	X				329
Group 1	LA8	141	147	1.85	X				329
	LA9	134	139	1.2		X	NO	YES	329
	LA10	131	135	1.3		X	NO	YES	329
	LA11	126	130	1.2	X				329
Alice Arm									
	LA12	131	135			X	NO	YES	388
	LA13	150	158	2.55	X				388
Group 2	LA14	144	150	2.05	X				388
	LA15	140	145	1.5		X	NO	YES	388
	LA16	135	140	1.4		X	NO	YES	388
	LA17	142	148	1.95	X				388
Hastings Arm									
Group 3	LA18	137	142	1.35		X	YES	NO	238
	LA19	161	170	3.15	X				245
	LA20	173	184	2.8	X				360
	LA21	152	160	2.2	X				274
	LA22	121	124	1.05		X	YES	NO	305
	LA23	120	124	1.05		X	YES	NO	305

Table 2. Arsenic in Golden King Crab Tissues from Alice and Hastings Arms.

Group	Sample ID	Gills[a] mg kg^{-1}	Hepatopancreas[a] mg kg^{-1}	Muscle[a] mg kg^{-1}
1	LA 6	99	103	145
Alice Arm	7	79	80	94[b]
(near	8	80	86	120
outfall)	9	90	146	180
	10	83	89	170
	11	72	125	155
2	12	90	140	145
Alice Arm	13	80	91	120
(mid)	14	115	165	120
	15	155	169	220
	16	100	61	135
	17	110	115	185
3	18	145	165	200
Hastings	19	77	60	120
Arm	20	71	73	130
	21	74	52	120
	22	115[b]	105	190
	23	110[b]	120	240

[a]Means of duplicate determinations; dry-weight basis; before rejection of outliers
[b]Single determination.

Table 3. Means (standard deviations) for Arsenic (mg kg^{-1}) in Golden King Crab Tissues from Alice and Hastings Arms.

Site	Tissue: Gills	Tissue: Hepatopancreas	Tissue: Muscle	Overall Tissues
Alice Arm (near outfall)	83.6(9.3) a[1] (n=12)[2]	104.7(35.5) a (n=12)	148.5(28.5) a (n=11)	111.3(37.5) k[1]
Alice Arm (mid)	108.2(25.7) a (n=12)	123.4(41.5) a (n=12)	154.2(238.7) a (n=12)	128.6(40.0) k
Hastings Arm	97.6(29.8) a (n=11)	95.7(41.1) a (n=12)	166.7(48.9) a (n=12)	120.6(52.2) k
Overall Sites	96.4(24.7) x[1]	107.9(40.1) x	156.7(39.4) y	

[1] Means within a group of means, means within a column of means or means within a row of means followed by the same letter, do not differ significantly at $P \leq 0.05$;
[2] Number of unpaired data points after rejection of outliers by Chauvenet's criterion ($n_{max} = 12$).

Table 4. Cadmium in Golden King Crab Tissues from Alice and Hastings Arms.

Group	Sample I.D.	Gills[a] mg kg^{-1}	Hepatopancreas[a] mg kg^{-1}	Muscle[a] mg kg^{-1}
1	LA 6	136	33	0.94
Alice Arm	7	84.8	115	DL[b,c]
(near	8	68.1	47	0.15
outfall)	9	81.0	51.7	0.45
	10	124	53	0.51
	11	76.5	162	0.97
	LA 12	132	71.0	0.82
2	13	78.5	63.7	0.22
Alice Arm	14	101	147	0.63
(mid)	15	141	108	1.45
	16	108	32.0	0.64
	17	87.7	55.2	0.095
3	LA 18	111	52.2	0.90
Hastings	19	46.8	39.2	0.36
Arm	20	71.7	104	0.59
	21	57.5	91.0	0.08
	22	59.7	62.1	0.15
	23	100	99.4	0.98

[a] Means of duplicate determinations; dry weight basis;
[b] Below detection limit;
[c] Single determination.

Table 5. Means (standard deviation) for cadmium (mg kg^{-1}) in Golden King Crab Tissues from Alice and Hastings Arms.

Site	Tissue			Overall Tissues
	Gills	Hepatopancreas	Muscle	
Alice Arm (near outfall)	95.0(26.8) a[1] (n=12)[4]	77.0(51.7) a (n=12)	0.60(0.35) a (n=11)	60.9(52.4) k[1]
Alice Arm (mid)	107.8(24.7) a (n=12)	79.5(39.6) a (n=12)	0.63(0.50) a (n=12)	62.7(52.9) k
Hastings Arm	72.0(24.5) a (n=12)	74.6(26.1) a (n=12)	0.51(0.40) a (n=12)	48.4(40.4) k
Overall Sites	92.2(28.8) y[1]	77.0(39.4) z	0.58(0.42) x	

[1]Means within a group of means, means within a column or within a row, followed by the same letter, do not differ significantly at $p \leq 0.05$;
[2]Number of unpaired data points after rejection of outliers (n_{max}=12).

Table 6. Copper in golden King Crab tissues from Alice and Hastings Arms.

Group	Sample I.D.	Gills mg kg^{-1}	Hepatopancreas mg kg^{-1}	Muscle mg kg^{-1}
1	LA 6	145	291	22
Alice Arm	7	222	259	6[b]
(near	8	154	285	35
outfall)	9	132	536	18
	10	193	301	14
	11	96	1018	227
2	LA 12	161	307	40
Alice Arm	13	203	602	75
(mid)	14	136	383	61
	15	91	321	21
	16	270	370	39
	17	288	263	26
3	LA 18	128	910	74
Hastings	19	289	478	71
Arm	20	144	568	95
	21	251	508	41
	22	164	958	105
	23	110	605	63

[a]Means of duplicate determinations; dry-weight basis; before rejection of outliers;
[b]Single determination

Table 7. Means (standard deviations) for copper (mg kg^{-1}) in Golden King Crab Tissues from Alice and Hastings Arms.

Site	Tissue: Gills	Tissue: Hepatopancreas	Tissue: Muscle	Overall Tissues
Alice Arm (near outfall)	147.8(35.1) a[1] (n=11)[2]	448.2(297.) b (n=12)	17.9(7.5) a (n=8)	230.5(257.) k[1]
Alice Arm (mid)	192.9(78.1) a (n=12)	346.2(88.1) b (n=11)	24.7(5.6) a (n=8)	203.9(144.) k
Hastings Arm	187.2(71.8) a (n=11)	671.0(206.) c (n=12)	63.0(15.2) d (n=10)	325.5(299.) m
Overall Sites	176.4(66.2) y[1]	492.5(251.) z	37.3(23.3) x	

[1]Means within a group, column or row of means, followed by the same letter; do not differ significantly at $p \leq 0.05$.
[2]Number of unpaired data points after rejection of outliers (n_{max}=12).
[3]Significant at $p \leq 0.001$.

Table 8. Molybdenum in golden King Crab Tissues from Alice and Hastings Arms.

Group	Sample I.D.	Gills[a] mg kg^{-1}	Hepatopancreas[a] mg kg^{-1}	Muscle[a] mg kg^{-1}
1	LA 6	2.0	0.75	0.35
Alice Arm	7	1.5	0.6	0.3[b]
(near	8	1.0	0.85	(0.4)[c]
outfall)	9	1.0	1.0	0.85
	10	2.0	0.85	0.35
	11	0.95	2.0	0.8
2	LA 12	2.0	0.8	(0.3)[c]
Alice Arm	13	1.4	0.65	(0.35)[c]
(mid)	14	2.0	1.5	0.45
	15	4.0	2.15	0.75
	16	1.5	0.7	0.4
	17	2.5	1.4	(0.3)[c]
3	LA 18	1.0	1.5	(0.5)[c]
Hastings	19	0.7	0.45	0.45
Arm	20	0.85	0.6	0.45[c]
	21	0.95	0.45	(0.2)
	22	1.0	1.55	0.5
	3	1.0	1.3	0.55

[a]Means of duplicate determinations, dry-weight basis; before rejection of outliers;
[b]Single determination;
[c]Data used to calculate mean includes one value which is the instrumental detection limit.

Table 9. Means (standard deviation) for molybdenum (mg kg^{-1}) in Golden King Crab Tissues from Alice and Hastings Arms.

Site	Tissue: Gills	Tissue: Hepatopancreas	Tissue: Muscle	Overall Tissues
Alice Arm (near outfall)	1.41(0.52) b[1] (n=12)[2]	0.83(0.21) ab (n=11)	0.56(0.26) a (n=10)	0.96(0.51) k[1]
Alice Arm (mid)	2.23(0.97) c (n=12)	1.20(0.69) b (n=12)	0.45(0.08) a (n=8)	1.40(1.01) m
Hastings Arm	0.91(0.12) ab (n=11)	0.98(0.67) ab (n=12)	0.48(0.15) a (n=10)	0.80(0.46) k
Overall Sites	1.53(0.84) z[1]	1.01(0.58) y	0.50(0.18) x	

[1] Means within a group, column or row of means, followed by the same letter, do not differ significantly at $P \leq 0.05$.
[2] Number of unpaired data points after rejection of outliers (n_{max}=12).

Table 10. Lead in Golden King Crab Tissues from Alice and Hastings Arms.

Group	Sample I.D.	Gills mg kg^{-1}	Hepatopancreas mg kg^{-1}	Muscle mg kg^{-1}
1	LA 6	2.39	0.23	c
Alice Arm	7	1.47	0.15	c
(near outfall)	8	0.39	0.11	c
	9	0.50	0.31	c
	10	1.90	0.69	c
	11	0.26	0.10	c
	X	1.15* [d]	0.26 [d]	
	S	0.89 (77%)	0.22 (84%)	
2	12	1.41	0.10	c
Alice Arm	13	0.80	0.06	c
(mid)	14	1.82	0.13	c
	15	1.96	0.17	c
	16	0.96	0.03	c
	17	2.58	0.07	c
	X	1.59+ [d]	0.09 [d]	
	S	0.67 (42%)	0.05 (55%)	
3	18	c	c	c
Hastings	19	c	c	c
Arm	20	c	c	c
	21	c	c	c
	22	c	c	c
	23	c	c	c

[a] Data obtained using graphite furnace AAS;
[b] Means of duplicate determinations; dry-weight basis;
[c] Value below limit of detection (LOD). LOD for gills: 0.30 mg kg^{-1}; muscle: 0.15 mg kg^{-1}; hepatopancreas: 0.10 mg kg^{-1};
[d] Coefficient of variation;
* Significantly > LOD; $p<0.05$;
+ Significantly > LOD; $p<0.005$.

Table 11. Zinc in Golden King Crab Tissues from Alice and Hastings Arms.

Group	Sample I.D.	Gills mg kg^{-1}	Hepatopancreas mg kg^{-1}	Muscle mg kg^{-1}
1	LA 6	93.4	166	173[b]
Alice Arm	7	90.2	230	193[b]
(near	8	75.3	173	194
outfall)	9	82.9	217	176
	10	95.9	194	163
	11	83.9	357	152
2	LA 12	97.2	167	163
Alice Arm	13	78.9	201	189
(mid)	14	91.6	355	174
	15	91.3	281	146
	16	100	132	176
	17	90.4	176	177
3	LA 18	95.7	422	180
Hastings	19	75.6	275	185
Arm	20	67.0	442	176
	21	86.4	178	168
	22	90.1[b]	461	186
	23	82.6[b]	448	209

[a]Means of duplicate determinations; dry-weight basis; before rejection of outliers.
[b]Single determinations

Table 12. Means (standard deviations) for zinc (mg kg^{-1}) in Golden King Crab Tissues for Alice and Hastings Arms.

Site	Tissue			Overall Tissues
	Gills	Hepatopancreas	Muscle	
Alice Arm (near outfall)	86.6(7.8) a[1] (n=11)[2]	222.5(71.9) b (n=12)	173.5(19.5) b (n=11)	162.7(71.6) k[1]
Alice Arm (mid)	93.4(5.4) a (n=11)	218.5(82.3) b (n=12)	170.6(19.1) b (n=12)	162.7(70.7) k
Hastings Arm	82.9(11.3) a (n=11)	370.8(114.) c (n=12)	183.7(17.8) b (n=12)	216.2(137.) m
Overall Sites	87.6(9.3) x[1]	270.6(114.) z	176.0(19.1) y	

[1]Means within a group, column or row of means, followed by the same letter, do not differ significantly at $p \leq 0.05$;
[2]Number of unpaired data points after rejection of outliers ($n_{max} = 12$).

Table 13. Metal-Binding Protein and Lipid Concentrations in the Hepatopancreas of Lithodes aequispina from Alice and Hastings Arms.

Group	Sample I.D.	Mean MBP[a] g kg^{-1}	Mean Lipid (%) A	B[b]
1	LA 6	16.6	16.7	16.3
Alice Arm	7	10.5	-	28.4
(outfall)	8	15.2	-	21.9
	9	9.5	30.2	27.9
	10	11.4	26.5	-
	11	18.8	11.0	10.6
	X	13.7[c]		
	S	3.7 (27.0%)[d]		
2	LA 12	11.8	-	30.1
Alice Arm	13	12.1	-	24.5
(mid)	14	15.8	-	14.4
	15	15.7	-	19.8
	16	11.6	27.3	-
	17	12.3	-	27.7
	X	13.2		
	S	2.0 (15.2%)		
3	LA 18	17.8	-	-
Hastings Arm	19	10.6	-	-
	20	13.4	-	-
	21	6.8	45.2	44.3
	22	16.3	16.8	-
	23	17.3	18.3	-
	X	13.8		
	S	4.4 (31.2%)		
	X_{Grand}	13.6		
	S_{Grand}	3.3 (24.4%)		

[a] Concentration of MBP determined by differential pulse polarography after denaturation of crude cytosol in 1:1 ETOH. Mean of duplicate determinations

[b] A and B are duplicate samples from the original collection of samples

[c] One standard deviation

[d] Relative standard deviation

Table 14. Standard Reference Material Data for Metals in this Study.

	n	As	Cd	Cu	Mo	Pb	Zn
SRM 1577 Bovine Liver	8	DL[b]	0.31±0.09[a]	185±14	2.63±0.52	DL[b]	1.37±0.9
Cert.value		(0.055)[c]	0.27±0.04	193±10	(3.2)	0.34±0.08	130±10
TORT-1 Lobster Hepatopancreas	10	30.9±3.2	26.1±1.0	374±17	1.7±0.48	9.9±2.0	166±6
Cert.value		24.6±2.2	26.3±2.1	439±22	1.5±0.3	10.4±2.0	177±10
SRM 1566 Oyster	11	14.5±4.2	3.60±0.08	59.5±2.5	DL	DL	861±12[e]
cert.value		13.4±1.9	3.5 ±0.4	63±3.5	(≤0.2))	0.48±0.04	852±14

[a] mean ± 1 standard deviation
[b] at or below detection limit for the method (ICPS)
[c] non-certified value
[d] certified values ± 95% confidence limits
[e] n = 10

Table 15. Calculated ICPS Detection Limits

Element	Mean Conc. in sol'n (mg L^{-1} ±1s)			Method LOD Range (mg kg^{-1})[b]					
	Blank[a]	Reference Material		Gill			Muscle		Hepatopancreas
As	0.2	0.38 ± 0.15[c]	[d]	9.3	±	18.0	4.8 ± 9.0	3.3	± 6.0
Cd	0.002	0.0069 ± 0.0033		0.10	±	0.40	0.06 ± 0.20	0.04	± 0.13
Cu	0.005	1.58 ± 0.16[c]		0.24	±	19.2	0.12 ± 9.2	0.084	± 6.4
Mo	0.01	0.046 ± 0.019[e]		0.60	±	2.3	0.30 ± 1.1	0.20	± 0.76
Pb	0.05	0.27 ± 0.10[e]		2.3	±	12	1.1 ± 6.0	0.75	± 4.0
Zn	0.005	4.43 ± 0.91		0.24	±	108	0.12 ± 55	0.084	± 27

[a] Reported 'less-than' values for fifteen reagent blank analyses;
[b] Based upon nominal 0.5, 1.0 and 1.5 g samples of gill, muscle and hepatopancreas tissue respectively, in a volume of 20 ml;
[c] From SRM 1566 data;
[d] From SRM 1577 data;
[e] From TORT-1 data.

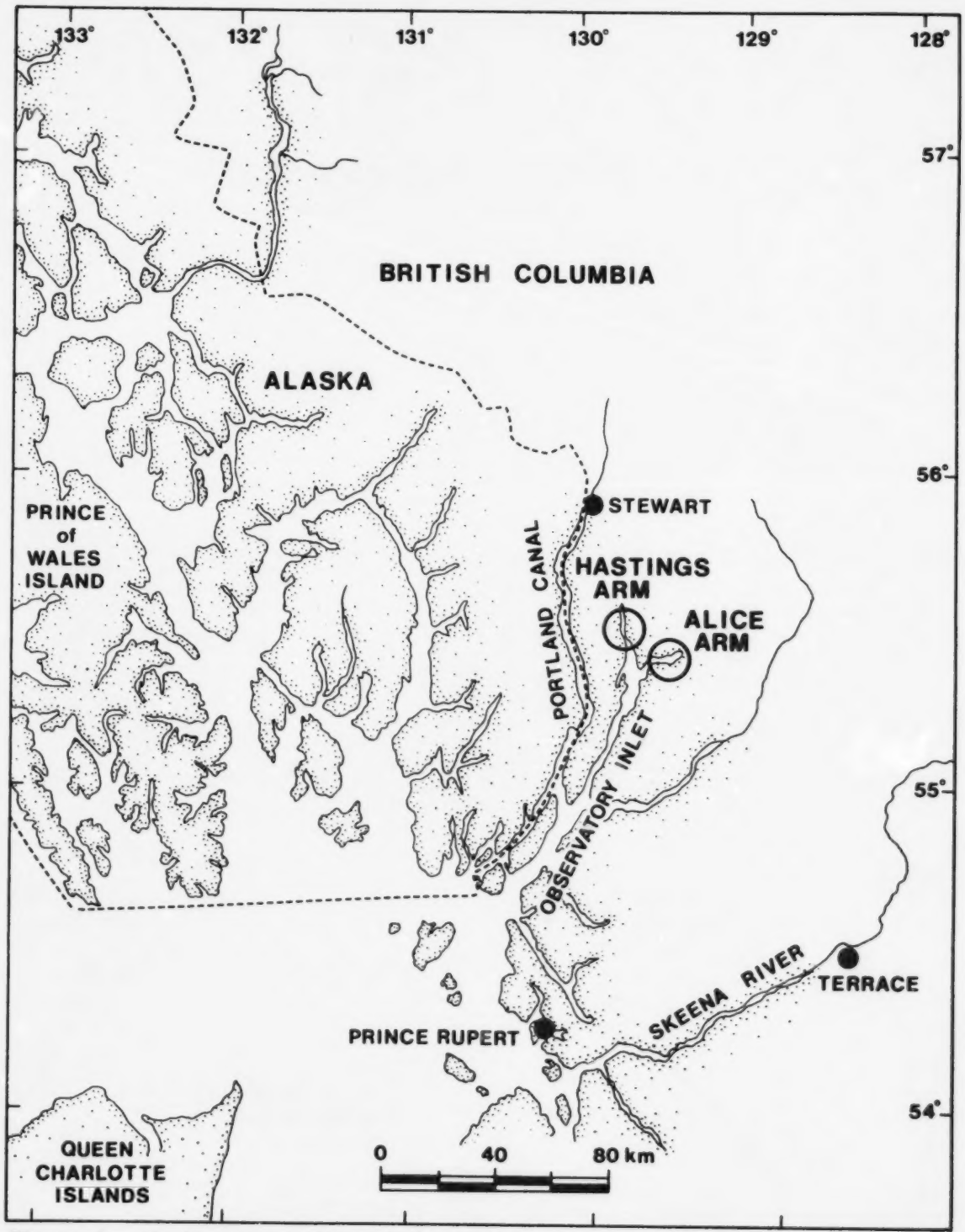


Figure 1. Study areas (circles) in Northern British Columbia

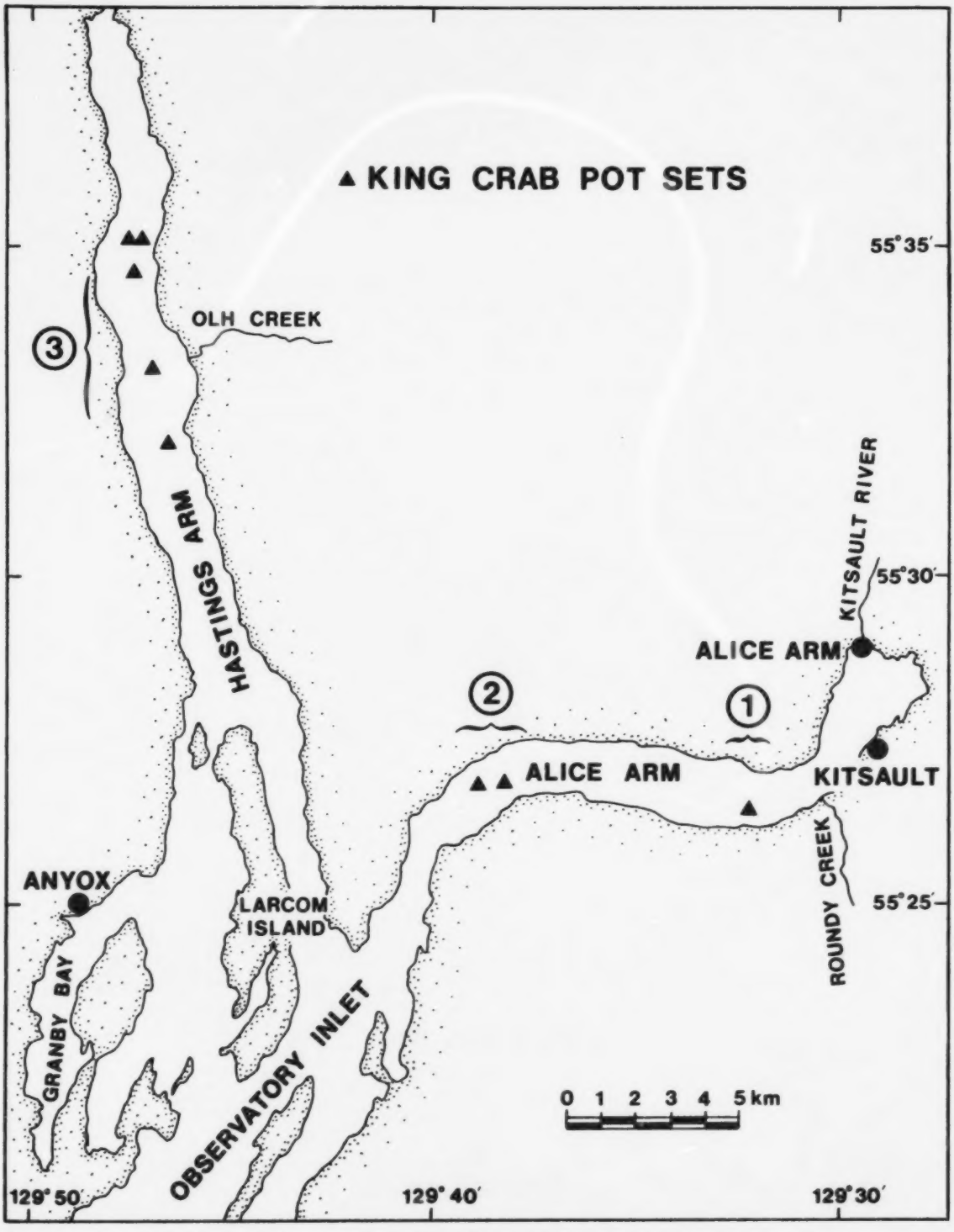


Figure 2. King Crab pot stations in Alice Arm and Hastings Arm, B.C.

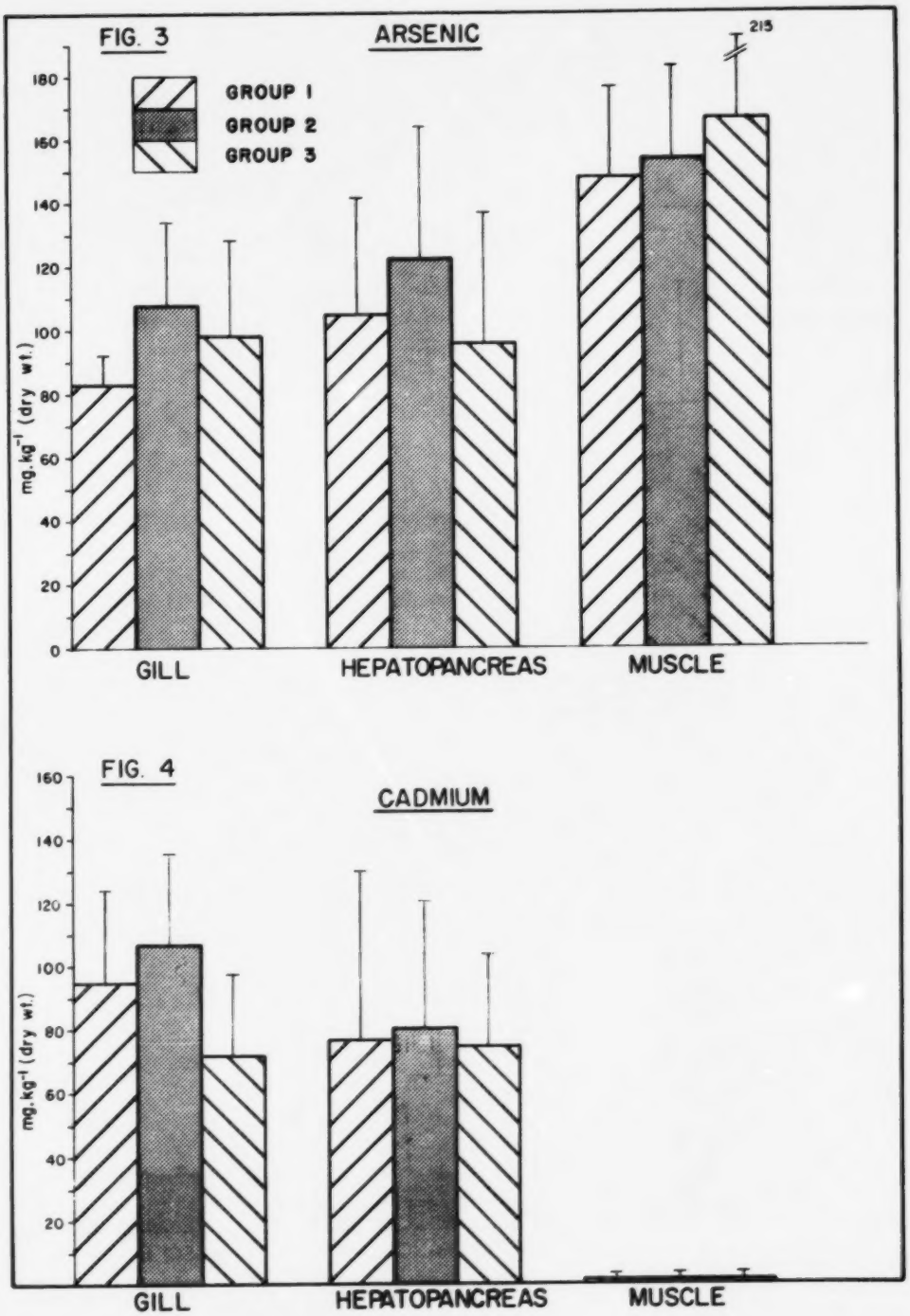


Figure 3. Arsenic concentrations in tissues of Lithodes aequispina from three sampled areas. Group 1: Alice Arm (near outfall), Group 2: Alice Arm (mid), Group 3: Hastings Arm.

Figure 4. Cadmium concentrations in tissues of Lithodes aequispina. See Fig. 3 for key to Groups.

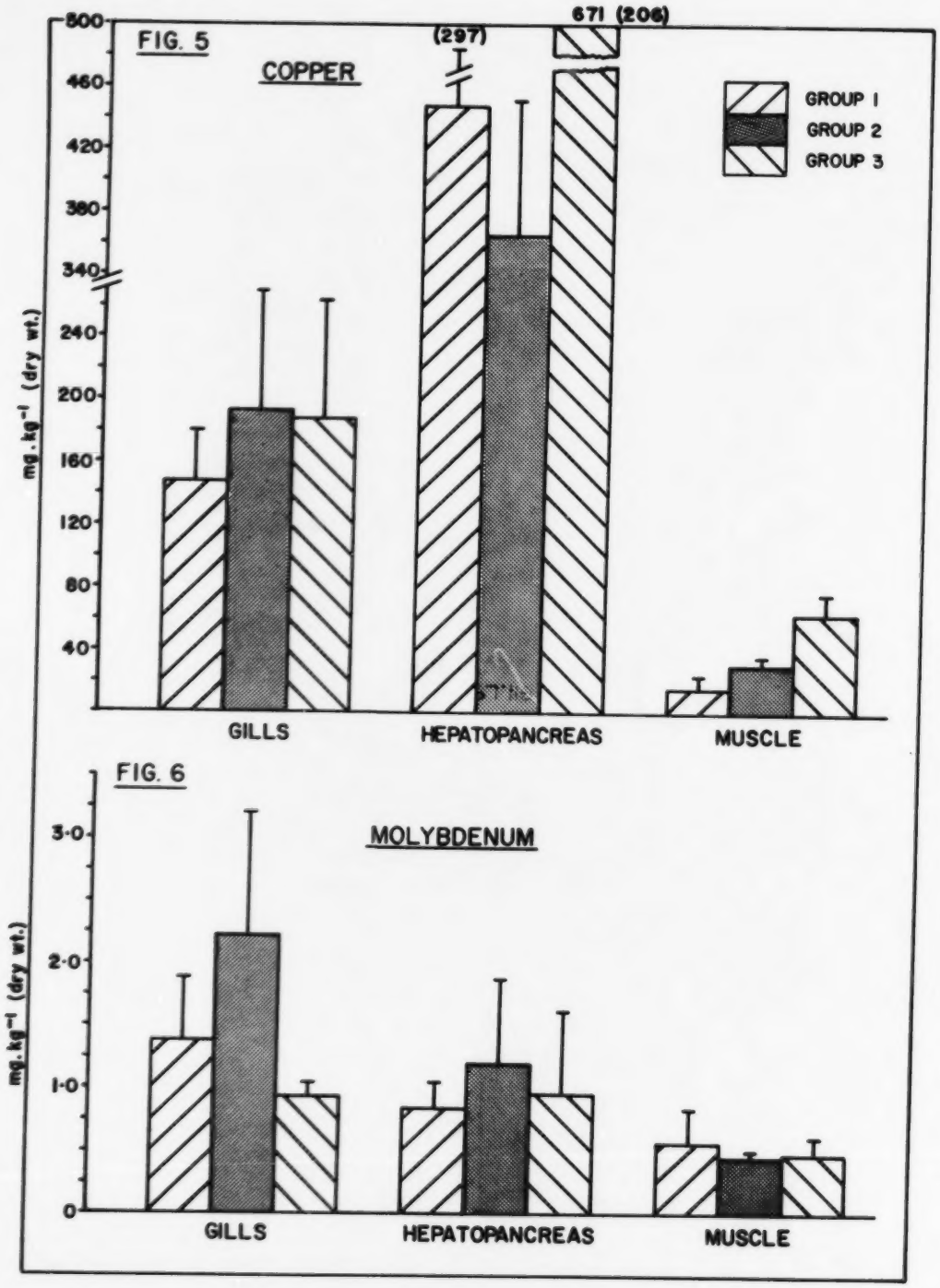
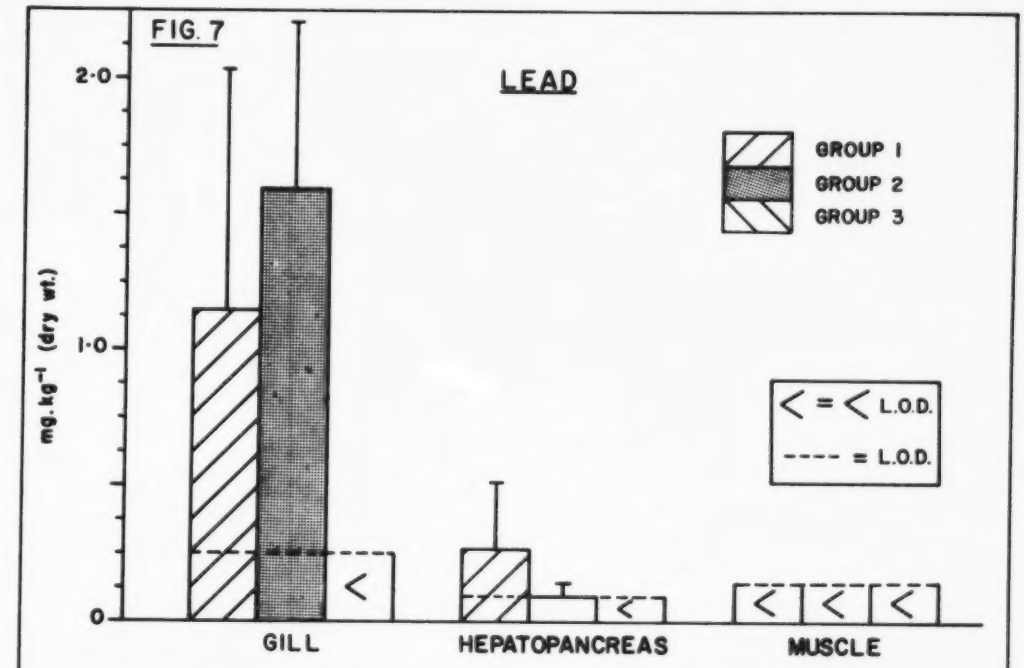


Figure 5. Copper concentrations in tissues of Lithodes aequispina. Key as described in Fig. 3.

Figure 6. Molybdenum concentrations in tissues of Lithodes aequispina. Key as described in Fig. 3.



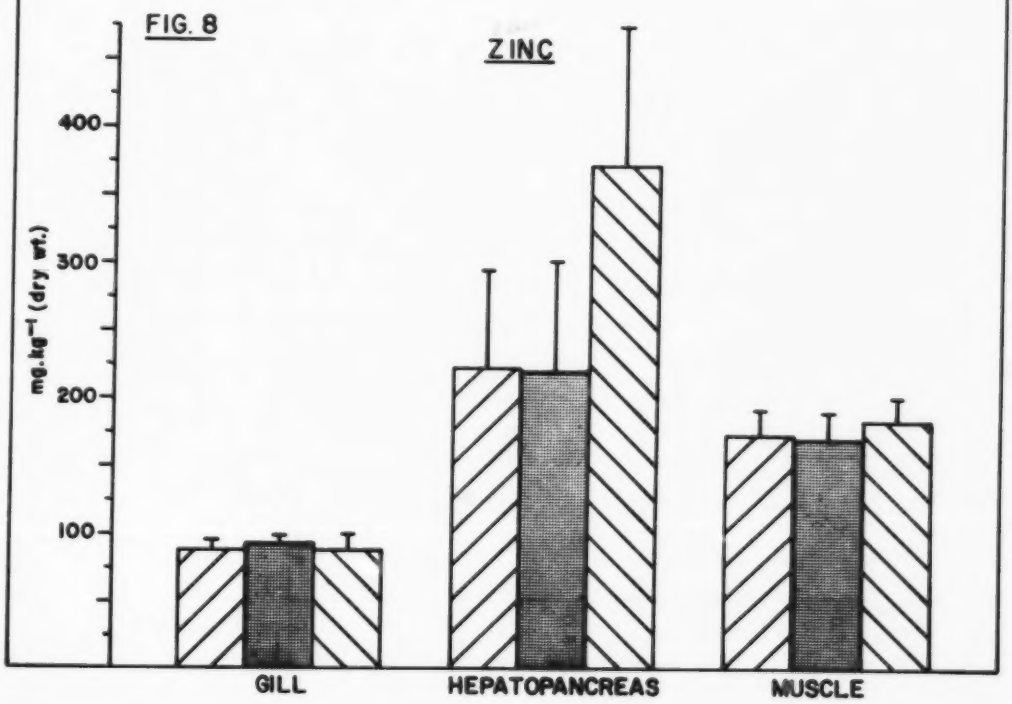


Figure 7. Lead concentrations in tissues of Lithodes aequispina. Key as described in Fig. 3.

Figure 8. Zinc concentrations in tisssues of Lithodes aequispina. Key as described in Fig. 3.

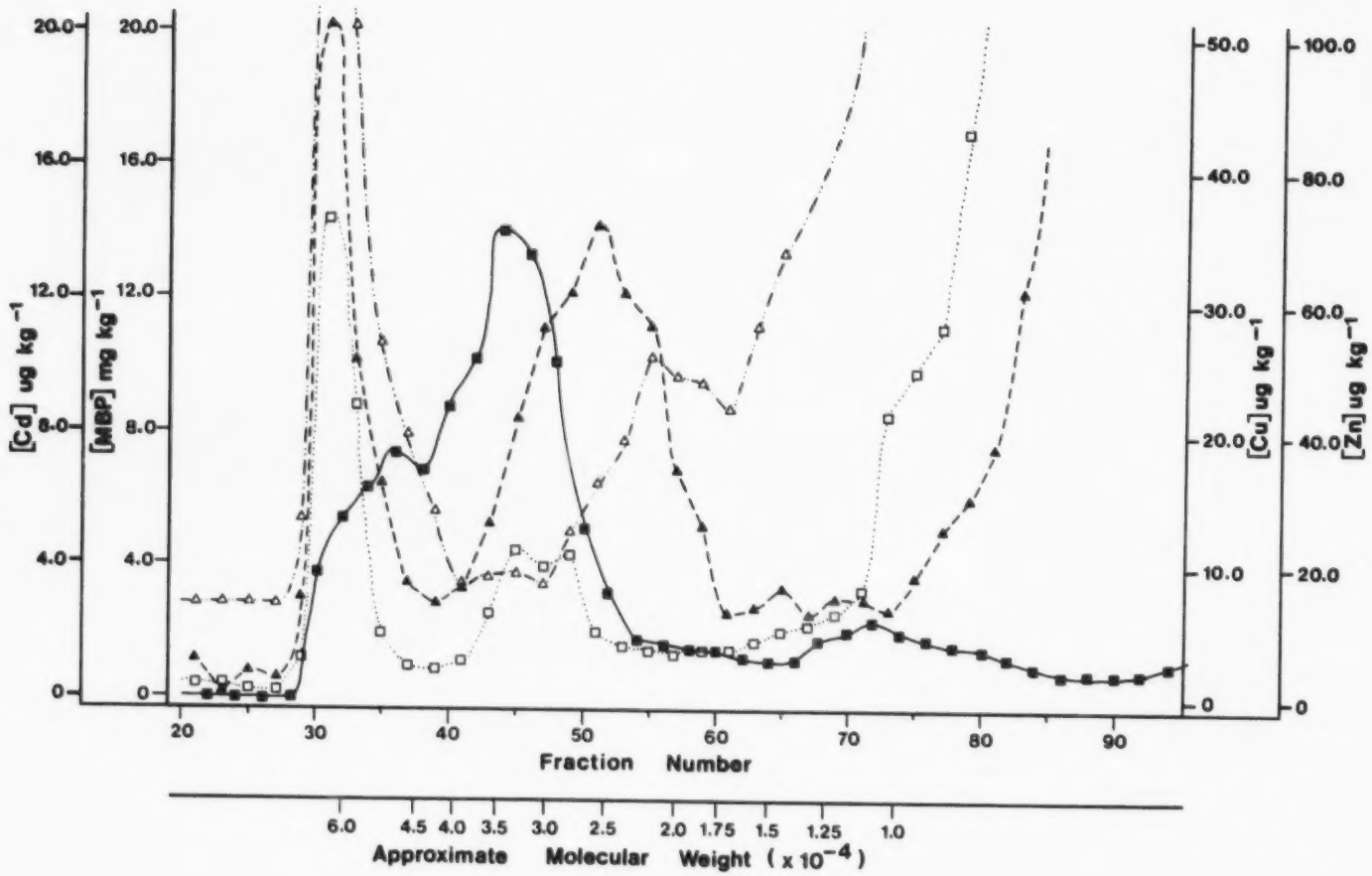


Figure 9. Sephadex G-75 elution profiles of non-denatured hepatopancreatic cytosol from Lithodes aequispina. Metal-binding protein (MBP):—■—■—; Cu:-·-△-·-△-·-; Cd:··□·· □··; Zn:-▲--▲--.

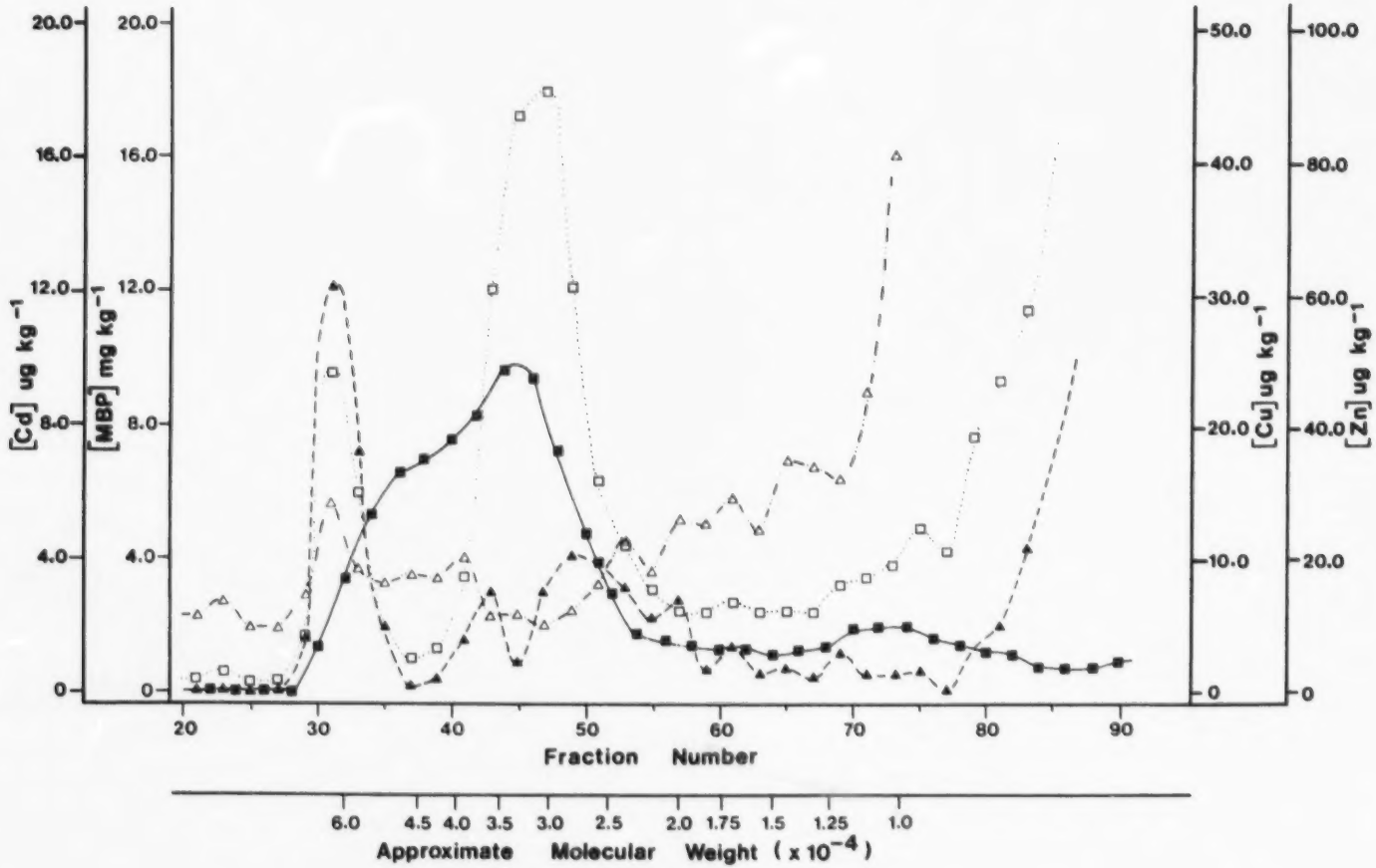


Figure 10. Sephadex G-75 elution profiles of ethanol denatured hepatopancreatic cytosol from Lithodes aequispina. Key to symbols as in Fig. 9.